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Influences of hydration on post-exercise cardiovascular control in humans

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Dehydration is known to decrease orthostatic tolerance and cause tachycardia, but little is known about the cardiovascular control mechanisms involved. To test the hypothesis that arterial baroreflex sensitivity increases during exercise-induced dehydration, we assessed arterial baroreflex responsiveness in 13 healthy subjects (protocol 1) at baseline (PRE-EX) and 1 h after (EX-DEH) 90 min of exercise to cause dehydration, and after subsequent intravenous rehydration with saline (EX-REH). Six of these subjects were studied a second time (protocol 2) with intravenous saline during exercise to prevent dehydration. We measured heart rate, central venous pressure and arterial pressure during all trials, and muscle sympathetic nerve activity (MSNA) during the post-exercise trials. Baroreflex responses were assessed using sequential boluses of nitroprusside and phenylephrine (modified Oxford technique). After exercise in protocol 1 (EX-DEH), resting blood pressure was decreased and resting heart rate was increased. Cardiac baroreflex gain, assessed as the responsiveness of heart rate or R–R interval to changes in systolic pressure, was diminished in the EX-DEH condition (9.17 ± 1.06 ms mmHg^{-1} *vs.* PRE-EX: 18.68 ± 2.22 ms mmHg^{-1}, $P < 0.05$). Saline infusion after exercise did not alter the increase in HR post-exercise or the decrease in baroreflex gain (EX-REH: 10.20 ± 1.43 ms mmHg^{-1}; $P > 0.10$ *vs.* EX-DEH). Saline infusion during exercise (protocol 2) resulted in less of a post-exercise decrease in blood pressure and a smaller change in cardiac baroreflex sensitivity. Saline infusion caused a decrease in MSNA in protocol 1. We conclude that exercise-induced dehydration causes post-exercise changes in the baroreflex control of blood pressure that may contribute to, rather than offset, orthostatic intolerance.

Dehydration in humans has several adverse effects on cardiovascular function, including a decrease in orthostatic tolerance and a relative tachycardia at rest and during exercise. Mild to moderate dehydration is common during prolonged exercise (Greenleaf, 1992; Sawka & Greenleaf, 1992). Even mild post-exercise dehydration (~ 1 % of body weight) increases resting heart rate (HR) and decreased tolerance to lower body negative pressure (Davis & Fortney, 1997), implying impaired cardiovascular control. Other forms of dehydration, such as those due to prolonged bed rest (Vernikos & Convertino, 1994; Kamiya *et al.* 1999; Custaud *et al.* 2002) and diuretic use (Frey *et al.* 1994) are also associated with decreases in orthostatic tolerance. The role of the arterial baroreflex in these cardiovascular impairments associated with dehydration is unclear.

Central venous pressure (CVP) has been shown to influence the arterial baroreflex control of HR. Previous reports document increased arterial baroreflex sensitivity with decreased CVP (Pawelczyk & Raven, 1989; Crandall *et al.*

1994) and decreases in arterial baroreflex sensitivity when CVP is increased (Shi *et al.* 1993). It has been proposed that these effects are due to the activation of cardiopulmonary baroreceptors with changes in CVP. However, some controversy exists regarding the role of cardiopulmonary reflexes in the effects of changes in central blood volume on the arterial baroreflexes (Taylor *et al.* 1995).

Although prolonged exercise often causes dehydration, the influences of the lower CVP on arterial baroreflex control may be different from those seen in previous studies (Pawelczyk & Raven, 1989; Shi *et al.* 1993; Crandall *et al.* 1994) since exercise itself has lasting effects on blood pressure regulation (Halliwill *et al.* 1996*a,b*). Furthermore, dehydration due to bed rest or diuretic use usually involves substantial decreases in plasma volume, whereas dehydration due to exercise is associated with smaller decreases in plasma volume because of post-exercise fluid shifts, which tend to restore intravascular volume (Jimenez *et al.* 1999; Hayes *et al.* 2000).

Table 1. Subject descriptive statistics (mean ± S.E.M.)

	Protocol 1	Protocol 2
n	13	6
Age (years)	25.9 ± 1.1	26.0 ± 1.8
Height (m)	1.79 ± 0.27	1.79 ± 0.34
Weight (kg)	78.4 ± 3.9	80.9 ± 6.4
$V_{O_2,peak}$ (ml kg^{-1} min^{-1})	39.8 ± 1.8	40.3 ± 3.5

The subjects who participated in protocol 2 were a subset of those who participated in protocol 1; they were not different in height, weight, age or $V_{O_2,peak}$ from the group as a whole ($P > 0.1$).

We hypothesised that (1) decreases in volume and CVP with exercise-induced dehydration would increase arterial baroreflex sensitivity and (2) increasing volume and CVP from the exercise-dehydrated condition would reverse this effect by decreasing arterial baroreflex sensitivity towards control values. To test these hypotheses, we assessed the baroreflex control of HR and muscle sympathetic nerve activity (MSNA) during exercise-induced dehydration and after volume infusion.

METHODS

General methods

Subjects. The protocol for these studies was approved by the Institutional Review Board of the Mayo Foundation. All studies were performed in accordance with the Declaration of Helsinki. Thirteen healthy young adults (mean age 25.9 ± 1.1 years; nine males, four females) gave their written informed consent to participate in these studies. Subjects were non-smokers and had no history of cardiovascular or other chronic diseases. They were taking no medication, including over-the-counter medications, with the exception of oral contraceptives. The descriptive data for this group of subjects are shown in Table 1. Subjects reported to the Mayo General Clinical Research Center (GCRC) on two occasions, a screening visit and the study day. Six (five males, one female) of the 13 subjects also participated in a second study day (protocol 2, described below). All studies were performed in a GCRC laboratory where ambient temperature was controlled at between 22 and 24 °C. All women were studied in the early follicular phase of the menstrual cycle or in the low hormone phase of oral contraceptives to minimise variability in the autonomic control of cardiovascular variables due to reproductive hormone status (Minson *et al.* 2000; Charkoudian, 2001).

Measurements. HR was measured from a three-lead ECG. Arterial blood pressure (AP) was measured on a beat-by-beat basis by finger photoplethysmography (Finapres) and was verified regularly by automated sphygmomanometry on the contralateral arm. Internal temperature was measured using a commercially available digital sublingual thermometer (Filac, Allegromedical) during the baseline period corresponding to each baroreflex trial. CVP was measured by placement of a peripherally inserted central catheter (PICC) in an antecubital vein and advanced to the level of the superior vena cava. The location of the PICC line was verified by measurement of the distance from the antecubital fossa to the midclavicular line, and inserting the catheter to that length. All measurements of CVP were recorded with subjects in the supine position after having rested in that position for at least 30 min.

MSNA was measured by peroneal microneurography, as described by Sundlof & Wallin (1977). Multiunit post-ganglionic MSNA was recorded from the peroneal nerve posterior to the fibular head with a tungsten microelectrode. The recorded signal was amplified 100 000-fold, band-pass filtered (700–2000 Hz), rectified and integrated (resistance-capacitance integrator circuit, time constant 0.1 s) by a nerve-traffic analyser.

Protocols

Screening visit. On the screen day, subjects performed an incremental bicycle exercise test to exhaustion to determine peak O_2 uptake ($\dot{V}_{O_2peak}$). After a 5 min warm-up period of light cycling, the workload was increased by 20, 25 or 30 W every minute. Whole-body O_2 uptake ($\dot{V}_{O_2}$) was measured by using a breath-by-breath mass spectrometry system previously validated against the Douglas bag collection technique across a broad range of breathing frequencies (Proctor & Beck, 1996). All subjects reached subjective exhaustion (rating of perceived exertion = 19–20) within 8–12 min, and achieved a respiratory exchange ratio (rate of CO_2 production/$\dot{V}_{O_2}$) > 1.15, as well as a peak HR within 10 % of the age-predicted maximum.

Once $\dot{V}_{O_2peak}$ was measured, subjects rested for 15–20 min and then returned to the cycle ergometer for assessment of the workload corresponding to a steady state $\dot{V}_{O_2}$ of 55 % of $\dot{V}_{O_2peak}$. This was the workload that was then used on the study day for the 90 min exercise bout.

Protocol 1: Exercise-induced dehydration
Subjects reported to the laboratory between 7.00 and 8.00 a.m. on the study day, after having eaten a standardised breakfast consisting of a commercially available nutrition bar (PowerBar Harvest; PowerBar, Berkeley, CA, USA) containing 260 calories (where 1 cal = 4.18 J), 7 g protein, 45 g carbohydrates and 5 g fat, as well as 500 ml of water. Subjects reported normal hydration over the week prior to the study day, and did not exercise or drink alcohol within 24 h of the study, or drink caffeine the morning of the study.

Figure 1 shows the timeline for protocol 1. Subjects rested in a supine position during placement of the PICC and a regular 18-gauge intravenous catheter in the contralateral arm (for administration of nitroprusside and phenylephrine boluses), as well as placement of the ECG electrodes, Finapres cuff on the middle finger and automated sphygmomanometer cuff on the upper arm. Subjects then rested quietly for ~ 30 min before the start of data collection.

PRE-EX baroreflex trial. Each baroreflex trial consisted of a 5 min baseline period during which the ECG and AP were recorded continuously. AP was verified against arm cuff pressure before and after this 5 min period. This was followed by administration of a bolus of nitroprusside (100 μg) and 60 s later by a bolus of phenylephrine (150 μg; known as the modified Oxford technique). Data were collected for a further 2 min.

Exercise. Subjects were weighed in their shorts and T-shirt prior to exercise, then changed into a separate set of clothes for exercise. During exercise, they also wore a water-impermeable plastic garment over their shorts and T-shirt to increase heating and therefore promote sweating and dehydration. Subjects then exercised at the workload previously determined to be 55 % of their individual $\dot{V}_{O_2peak}$ for 90 min, with 5 min breaks every 30 min. The workload was sometimes decreased slightly (by ~ 5–10 W) during the last 30–45 min based on reported subjective fatigue. AP (measured by sphygmomanometer on the arm) and HR were

recorded every 10 min during exercise. After exercise, the subjects dried off and changed back to the original shorts and T-shirt in which they had been weighed for the post-exercise measurement of body weight.

EX-DEH baroreflex trial. After measurement of their post-exercise body weight, subjects rested supine for 45–60 min to allow for equilibration of body fluids. In eight subjects, this time was also used to prepare for microneurography measurements at the peroneal nerve. After this rest period, recording of a 5 min baseline and modified Oxford protocol were performed as described earlier.

Intravenous rehydration. After the EX-DEH baroreflex trial, a volume of saline equivalent to the amount of body weight lost during exercise was infused over 20–25 min via the antecubital intravenous catheter.

EX-REH baroreflex trial. Following intravenous rehydration, a third baroreflex trial was conducted as described above. The subject was then de-instrumented, their final body weight was measured to ensure the subject had been completely rehydrated, and the subject was given lunch and discharged from the GCRC.

Protocol 2: Exercise with simultaneous intravenous saline
In order to assess more comprehensively the influence of hydration status in our studies, we included a second protocol to assess the effect of intravenous saline given during exercise instead of after exercise. Thus, six of the subjects who had completed protocol 1 returned to the laboratory to take part in protocol 2. Protocol 2 was identical to protocol 1 except that saline was given intravenously during the exercise. The amount of saline was calculated based on the body weight lost during the individual subject's participation in protocol 1, and the infusion rate was controlled such that the total amount of saline was given steadily starting at 10 min into the exercise (when the subject had started to sweat) until the end of exercise. Baroreflex trials were conducted as in protocol 1 before exercise (PRE-EX), after exercise + saline infusion (EX-HY1) and again after 25 min (EX-HY2). In protocol 2, EX-HY1 and EX-HY2 baroreflex trials were separated by 25 min (as with the second two baroreflex trials in protocol 1), but in this protocol no further saline was given; the third assessment (EX-HY2) was used as a time control for comparison to protocol 1.

Blood samples
In order to assess plasma volume changes, electrolyte balance and hormonal responses during each trial, blood samples were drawn from the antecubital venous catheter at three time points corresponding to the three baroreflex trials. For all blood samples, subjects had been resting supine for a minimum of 30 min before the samples were drawn. Samples were analysed for haemoglobin, haematocrit, osmolality, Na^+, K^+, catecholamines and plasma renin activity.

Data analysis
HR, AP, CVP and MSNA were sampled at 250 Hz using data acquisition software (Windaq, Dataq Instruments, Akron, OH, USA) and stored on a personal computer for offline analysis. Data were analysed using Windaq signal processing software.

The extent of dehydration was assessed by subtracting post-exercise body weight from the value measured prior to exercise, and calculated as a percent of body weight. Changes in plasma volume were calculated from haemoglobin concentration and haematocrit using the method of Dill & Costill (1974).

MSNA was quantified as total integrated activity, which was defined as the summed area under the curve of the bursts of MSNA. Each MSNA recording was normalised by assigning the largest sympathetic burst an amplitude of 1000. All other bursts for a particular study were calibrated against that value (Halliwill, 2000). The zero nerve activity level was determined from the mean voltage during a period of neural silence between sympathetic bursts. A period in which bursts were absent for > 6 s was found in each tracing and used for this purpose.

Assessment of baroreflex control of HR and MSNA. We used sequential boluses of nitroprusside (100 μg) and phenylephrine (150 μg) to decrease and increase, respectively, AP.

We assessed cardiac baroreflex sensitivity using the relationship between R–R interval (RRI) and systolic blood pressure (SBP) during vasoactive drug boluses (Minson *et al.* 2000; Halliwill & Minson, 2002). The slope of this relationship was used as an index of baroreflex sensitivity. The operating point for the relationship in terms of resting AP and HR was determined as the average values over the 5 min period immediately preceding the nitroprusside bolus. Values for RRI from baroreflex trials were pooled over 2 mmHg ranges for analysis to minimise variability due to non-baroreflex influences such as respiration (Halliwill & Minson, 2002).

Analysis of baroreflex sensitivity in terms of RRI gives results that are directly related to efferent vagal activity to the heart (Parker *et al.* 1984), whereas HR is linearly related to cardiac output and AP. The reciprocal relationship between HR and RRI can result in a decrease in RRI slope with changes in baseline HR due to the fact that (mathematically) a given change in HR results in less of a change in RRI when baseline HR is higher (O'Leary, 1996). In order to be comprehensive in our analysis, we analysed the present data in terms of both RRI and HR.
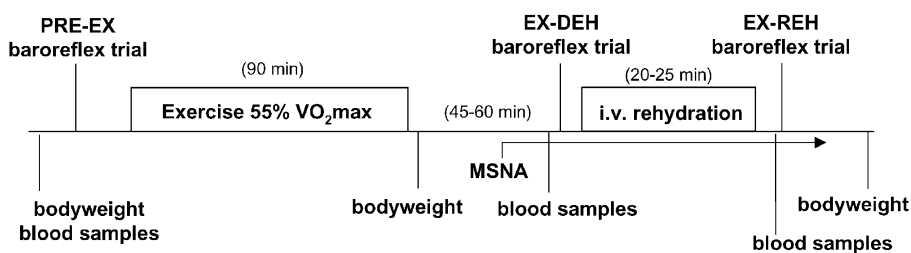
Figure 1. Timeline for protocol 1
Each baroreflex trial consisted of 5 min of rest; then a bolus of sodium nitroprusside was administered, followed 1 min later by a bolus of phenylephrine.

Table 2. Blood values corresponding to each of the three baroreflex trial time points

Protocol 1	Trial PRE-EX	EX-DEH	EX-REH
Haemoglobin (g dl^{-1})	13.4 ± 0.3	13.6 ± 0.4 ‡	12.85 ± 0.3 *
Haematocrit (%)	38.9 ± 0.8	39.3 ± 0.9 ‡	37.2 ± 0.8 *
Osmolality (mosmol kg^{-1})	286 ± 1	289 ± 1 *	290 ± 1 *
Na$^+$ (mEq l^{-1})	138 ± 1	140 ± 0 *	139 ± 0 *
K$^+$ (mEq l^{-1})	4.1 ± 0.1	4.6 ± 0.1 *	4.4 ± 0.1 *
Noradrenaline (pg ml^{-1})	194 ± 26	226 ± 22 †	241 ± 25 †
Adrenaline (pg ml^{-1})	20 ± 2	33 ± 4 *	22 ± 2
Plasma renin activity (ng AngI ml^{-1} h^{-1})	0.79 ± 0.10	2.58 ± 0.45 *	1.41 ± 0.23
Protocol 2	PRE-EX	EX-HY1	EX-HY2
Haemoglobin (g dl^{-1})	13.2 ± 0.4	12.5 ± 0.3 *	12.7 ± 0.4 *
Haematocrit (%)	38.2 ± 1.0	35.9 ± 1.0 *	36.4 ± 1.0 *
Osmolality (mosmol kg^{-1})	287 ± 0	291 ± 2	291 ± 1
Na$^+$ (mEq l^{-1})	138 ± 1	139 ± 1	138 ± 0
K$^+$ (mEq l^{-1})	4.0 ± 0.10	4.4 ± 0.1 *	4.8 ± 0.2 *
Noradrenaline (pg ml^{-1})	200 ± 34	174 ± 27	175 ± 27
Adrenaline (pg ml^{-1})	24 ± 3	27 ± 3	22 ± 3
Plasma renin activity (ng AngI ml^{-1} h^{-1})	0.69 ± 0.19	1.24 ± 0.44	1.07 ± 0.30

AngI, Angiotensin I. Protocol 1: * $P < 0.05$ *vs.* PRE-EX, † $P = 0.05$ *vs.* PRE-EX and ‡ $P < 0.05$ *vs.* EX-REH.
Protocol 2: * $P < 0.05$ *vs.* PRE-EX

An index of baroreflex control of sympathetic outflow was provided by the relationship between MSNA and diastolic blood pressure (DBP) during the drug boluses (Halliwill *et al.* 1996*a*; Halliwill & Minson, 2002). To perform a linear regression between MSNA and pressure, values for MSNA from baroreflex trials were first signal-averaged over 3 mmHg pressure ranges ('bins') via custom-designed software, as described previously (Halliwill, 2000; Halliwill & Minson, 2002). This pooling procedure reduces the statistical impact of the inherent beat-by-beat variability in nerve activity due to non-baroreflex influences (e.g. respiration). A window of nerve activity that was 2.0 s in length and synchronised by the R wave of the electrocardiogram was signal averaged. The window was time shifted to account for the latency between R waves and sympathetic bursts. The duration of the shift was varied as needed from subject to subject, but averaged 1.3 s.

The slope of the linear portion of the relationship between MSNA and DBP was used as an index of reflex sensitivity. The operating point for the relationship in terms of resting AP and nerve activity was assessed as the averaged values over the 5 min period immediately preceding the nitroprusside bolus. DBP was used because MSNA correlates closely with DBP but not with SBP (Sundlof & Wallin, 1977).

Statistical analysis

Data are presented as means ± S.E.M. and were analysed statistically using SigmaStat software (SPSS, San Rafael, CA, USA). Baseline values for HR, SBP, DBP and all blood concentrations measured, as well as cardiac baroreflex sensitivity, were analysed across conditions using a one-way repeated-measures analysis of variance (ANOVA). When significance was detected, Tukey's *post-hoc* test was used to identify individual differences. When only two conditions were being compared (e.g. sensitivity of MSNA baroreflex between the two post-exercise baroreflex trials), Student's *t* test was used. Statistical significance was accepted at $P < 0.05$.

RESULTS

Extent of dehydration

Protocol 1. Body weight decreased from 77.5 ± 3.8 to 76.3 ± 3.7 kg ($P < 0.01$) from PRE-EX to EX-DEH in protocol 1. Average fluid loss was 1.2 ± 0.1 kg, or 1.6 ± 0.1 % of body weight. Saline infusion restored body weight to pre-exercise levels (77.6 ± 3.8 kg; $P > 0.1$ *vs.* pre-exercise levels). CVP ($n = 5$) decreased from 5.0 ± 0.9 mmHg at the PRE-EX trial to 3.0 ± 0.3 mmHg post-exercise at the EX-DEH trial ($P < 0.05$ *vs.* PRE-EX), then increased to 6.3 ± 0.6 mmHg at the EX-REH trial ($P < 0.05$ *vs.* PRE-EX and EX-DEH). The average calculated change from baseline in plasma volume at the EX-DEH trial was −1.9 ± 1.1 %, and at the EX-REH trial it was +7.4 ± 1.7 %.

Protocol 2. As expected, saline infusion during exercise prevented dehydration in protocol 2. Before exercise, body weight was 79.9 ± 6.3 kg; post-exercise this value was 79.9 ± 6.1 kg ($P > 0.1$). Intravenous saline during exercise also prevented the post-exercise decrease in CVP (PRE-EX: 3.4 ± 0.9; EX-HY1: 3.0 ± 0.7; EX-HY2: 2.7 ± 0.7 mmHg; $P > 0.1$; $n = 5$). The average calculated change in plasma volume from baseline in protocol 2 was + 9.7 ± 3.0 % at the EX-HY1 trial and + 7.2 ± 2.2 % at the EX-HY2 trial.

Blood values and temperature

Values for haemoglobin, haematocrit, plasma osmolality, Na$^+$, K$^+$, catecholamines and plasma renin activity are shown in Table 2. As expected, indicators of dehydration such as plasma osmolality, renin activity and cate-cholamines increased in the EX-DEH trial of protocol 1. In

protocol 2, where dehydration was prevented during exercise, renin activity and catecholamines were not different among trials. However, plasma osmolality demonstrated a trend towards an increase ($P = 0.06$) in the EX-HY1 and EX-HY2 trials of protocol 2. One hour after exercise, internal body temperature remained elevated: $0.47 \pm 0.12\,°C$ higher in EX-DEH compared to PRE-EX in protocol 1, and $0.56 \pm 0.10\,°C$ higher in PRE-EX compared to EX-HY1 trials in protocol 2 ($P > 0.5$ for deltas, protocol 1 *vs.* protocol 2).

Resting 'operating point' values for HR and AP

Protocol 1. Resting HR was increased in the post-exercise trials (see Fig. 2*A*). One of the most striking findings of the present study was that this increased resting HR was not altered by the volume infusion that corrected the post-exercise drop in blood pressure and increased CVP.

Results for resting AP are shown in Table 3. Resting SBP, DBP and mean AP decreased in EX-DEH compared to PRE-EX. This post-exercise hypotension was reversed by intravenous saline rehydration such that in the EX-REH trial, AP was not significantly different from the PRE-EX trial ($P > 0.05$).

Protocol 2. Consistent with the data from protocol 1, saline infusion during exercise did not prevent or alter the rise in HR seen post-exercise (see Fig. 2*B*). The increase in HR from PRE-EX to EX-DEH was 15 ± 2 beats min^{-1} in protocol 1, and 13 ± 3 beats min^{-1} from PRE-EX to EX-HY1 in protocol 2 ($P > 0.10$ for deltas, protocol 1 *vs.* protocol 2).

Prevention of dehydration with intravenous saline during exercise minimised the post-exercise decrease in AP such that SBP, DBP and mean AP were not statistically different among trials in this protocol ($P > 0.05$; see Table 3).

Cardiac baroreflex sensitivity

Protocol 1. We analysed the sensitivity of the cardiac baro-reflex using both HR and RRI. With both analyses, the sensitivity, assessed as the slope of the linear portion of the relationship, decreased in the EX-DEH trial, and was not restored by saline infusion (EX-REH trial). All linear baroreflex relations demonstrated good linear regression correlation coefficients, with r^2 values ranging from 0.63 to 0.95 (most lying between 0.80 and 0.95).

The sensitivity of the cardiac baroreflex in terms of RRI was 18.68 ± 2.22 ms $mmHg^{-1}$ in the PRE-EX trial, and decreased to 9.17 ± 1.06 ms $mmHg^{-1}$ in the EX-DEH trial ($P < 0.05$). Saline infusion after exercise did not correct this decreased sensitivity, such that in the EX-REH trial, sensitivity was 10.2 ± 1.4 ms $mmHg^{-1}$ ($P > 0.1$ *vs.* EX-DEH).

In terms of HR, sensitivity decreased from -1.08 ± 0.08 beats min^{-1} $mmHg^{-1}$ at PRE-EX to -0.82 ± 0.07 beats min^{-1} $mmHg^{-1}$ at EX-DEH ($P < 0.05$). As with RRI sensitivity, no further change occurred with

Table 3. Resting values (mean ± S.E.M.) for arterial pressures (mmHg) prior to each of the three baroreflex trials for both protocols

Protocol 1	SBP	DBP	MAP
PRE-EX	141 ± 3	77 ± 2	98 ± 3
EX-DEH	$125 \pm 3\,^*$	$68 \pm 2\,^*$	$87 \pm 2\,^*$
EX-REH	137 ± 4	76 ± 4	96 ± 3
Protocol 2			
PRE-EX	130 ± 4	71 ± 3	91 ± 3
EX-HY1	121 ± 4	68 ± 2	86 ± 2
EX-HY2	124 ± 5	71 ± 2	89 ± 2

SBP, systolic blood pressure; DBP, diastolic blood pressure, MAP, mean arterial pressure. $^*P < 0.05$ *vs.* PRE-EX

post-exercise saline infusion (EX-REH: -0.86 ± 0.11 beats min^{-1} $mmHg^{-1}$; $P > 0.05$ *vs.* EX-DEH).

Figure 3 shows data from a representative subject for the linear portion of the baroreflex relationship between RRI
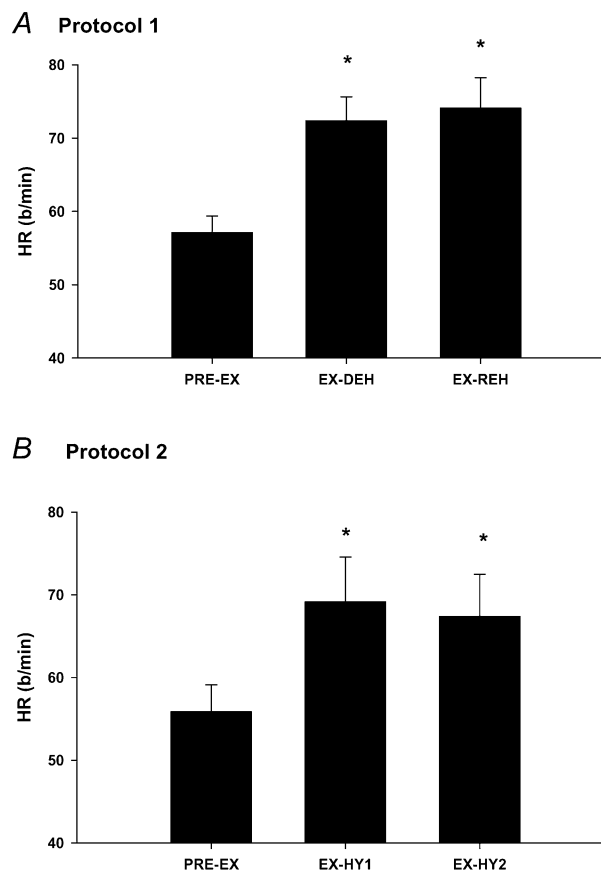

Figure 2. Average resting HR over the 5 min period prior to the administration of vasoactive drug boluses for each of the three baroreflex trials in both protocols

Average resting HR over the 5 min period prior to the administration of vasoactive drug boluses for each of the three baroreflex trials in protocol 1 (*A*, $n = 13$) and protocol 2 (*B*, $n = 6$). Note the increase in resting HR after exercise that is neither reversed by saline infusion in protocol 1 nor prevented by saline infusion during exercise in protocol 2. $^*P < 0.05$ *vs.* PRE-EX.

and SBP (*A*) and a graphical representation of the average regression equations (*n* = 13) for this relationship in the three baroreflex trials of protocol 1 (*B*).

Protocol 2. As with resting AP, the differences in sensitivity of the cardiac baroreflex were minimised when saline was infused during exercise in protocol 2. One subject was an outlier, with an exceptionally high RRI sensitivity of 45 ms mmHg^{-1}. Whether or not the data from this subject were included, values for cardiac baroreflex sensitivity were not significantly different among trials (*P* > 0.05 by ANOVA). However, the trend for decreased sensitivity in the post-exercise condition remained. When the outlier was excluded, the average RRI sensitivity for protocol 2 was 14.7 ± 2.5 ms mmHg^{-1} for PRE-EX, 11.06 ± 2.5 ms mmHg^{-1} for EX-HY1 and 11.52 ± 2.86 ms mmHg^{-1} for EX-HY2. Average HR sensitivity was −0.88 ± 0.11 beats min^{-1} mmHg^{-1} for PRE-EX, −0.80 ± 0.13 beats min^{-1} mmHg^{-1} for EX-HY1 and −0.84 ± 0.14 beats min^{-1} mmHg^{-1} for EX-HY2 (*P* > 0.05 by ANOVA).

Muscle sympathetic nerve activity

The design of the present study was such that we could examine changes in the control of MSNA in the post-exercise dehydrated state to the post-exercise hydrated

state, with saline having been given either during or after exercise. We measured MSNA in eight individuals in protocol 1, and in four individuals in protocol 2.

Protocol 1. MSNA is expressed as total integrated activity (units). MSNA during baseline for the EX-DEH trial in protocol 1 was 39 ± 6 units beat^{-1} or 2950 ± 515 units min^{-1}, and decreased at EX-REH to 25 ± 7 units beat^{-1} (*P* < 0.05) or 1859 ± 562 units min^{-1} (*P* < 0.05). The sensitivity of the baroreflex control of MSNA was −5.01 ± 1.26 units beat^{-1} mmHg^{-1} in the EX-DEH trial, and tended to decrease (*P* = 0.09) to −3.08 ± 0.66 units beat^{-1} mmHg^{-1} after saline infusion at EX-REH. In most subjects this sensitivity decreased slightly during EX-REH; a representative example of baroreflex control of MSNA from one subject is shown in Fig. 4*A*. Figure 4*B* is a graphical representation of the average regression equations (*n* = 8) for the linear portions of the MSNA baroreflex curves for the EX-DEH and EX-REH conditions of protocol 1.

Protocol 2. In protocol 2, there was no difference between EX-HY1 and EX-HY2 in resting MSNA or in the sensitivity of the MSNA baroreflex. Resting MSNA was 33 ± 7 units beat^{-1} or 2282 ± 585 units min^{-1} in EX-HY1, and 34 ± 10 units beat^{-1} or 2279 ± 753 units min^{-1} in
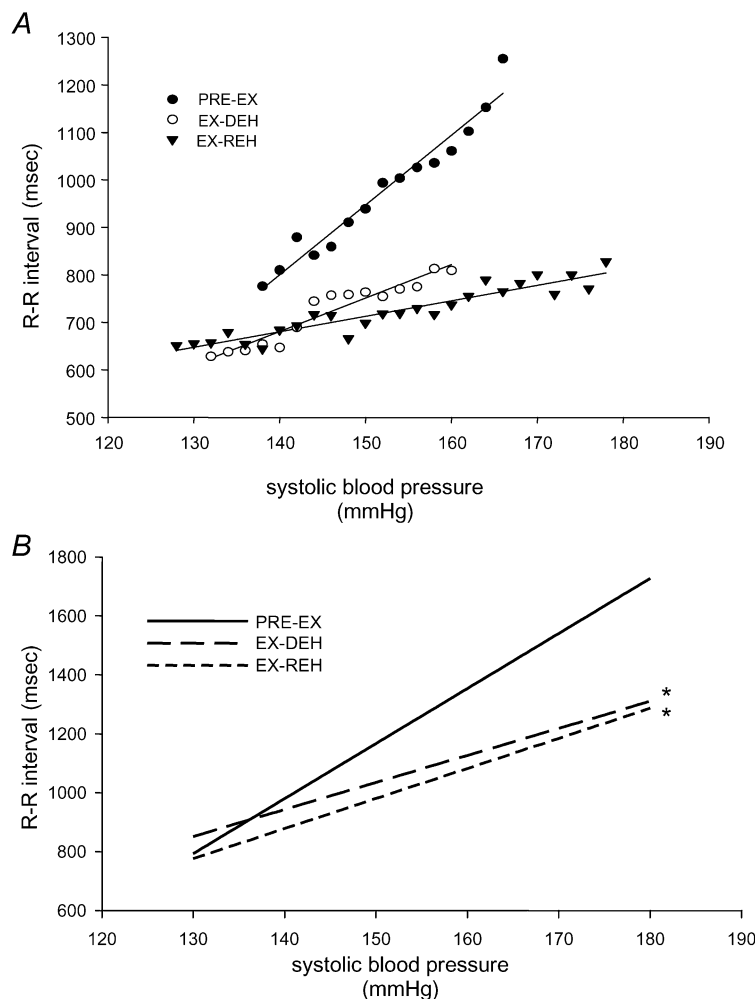
Figure 3. Relationship between RRI and systolic blood pressure in the EX-DEH and EX-REH trials of protocol 1

A, data from a representative subject for the three baroreflex trials of protocol 1. Graphs show the linear portion of cardiac baroreflex responses. Note the decrease in cardiac baroreflex sensitivity (slope) in the EX-DEH and EX-REH trials. *B*, graphical representation of averaged regression equations from the linear portion of the cardiac baroreflex curves for all subjects (*n* = 13) from protocol 1. *P* < 0.05 for slopes *vs.* PRE-EX.

EX-HY2 ($P > 0.5$ for both comparisons). The sensitivity of baroreflex control of MSNA was -2.72 ± 1.08 units beat^{-1} mmHg^{-1} in EX-HY1, and -2.48 ± 0.85 units beat^{-1} mmHg^{-1} in EX-HY2 ($P > 0.5$).

DISCUSSION

The main new finding of the present study is that the increased resting HR seen with exercise-induced dehydration was not responsive to a volume infusion that increased CVP and brought AP back to pre-exercise levels. We also observed a decrease in cardiac baroreflex sensitivity with exercise-induced dehydration in protocol 1. Although the trend for decreased baroreflex sensitivity post-exercise remained in protocol 2 (when intravenous saline prevented dehydration during exercise), it was smaller and not statistically significant. However, the increase in resting HR post-exercise was not different between protocols 1 and 2. This suggests a dissociation between the control of resting HR and cardiac baroreflex control in these studies.

The lack of decrease in resting HR with volume infusion may mean that the tachycardia seen after exercise is independent of baroreflex influences. For example, increased body temperature is known to increase the sinoatrial node rate (Gorman & Proppe, 1982, 1984; Crandall *et al.* 2003) and the increase in body temperature remaining at 1 h post-exercise in the present study ($\sim 0.5\,^{\circ}$C) might have contributed to the increased HR. However, it is important to note that larger increases in body temperature do not alter the sensitivity of arterial baroreflex control of HR (Gorman & Proppe, 1982; Crandall *et al.* 2003); thus, the effect on baroreflex sensitivity observed in the present study was probably not a result of the level of internal body temperature. Interestingly, a similar dissociation between blood pressure and HR was noted by Norton *et al.* (1999) in a study examining cardiovascular drift during prolonged exercise. These investigators noted that blood pressure fell progressively during 1 h of cycle exercise, and they were

Figure 4. Relationship between MSNA and diastolic blood pressure in the EX-DEH and EX-REH trials of protocol 1

A, data from a representative subject showing baroreflex control of MSNA in the EX-DEH and EX-REH trials of protocol 1. *B*, graphical representation of averaged regression equations ($n = 8$) from the linear portion of the baroreflex curves for MSNA during the EX-DEH and EX-REH trials of protocol 1. Overall, the sensitivity of baroreflex control of MSNA tended to be lower ($P = 0.09$) in EX-REH compared to EX-DEH.
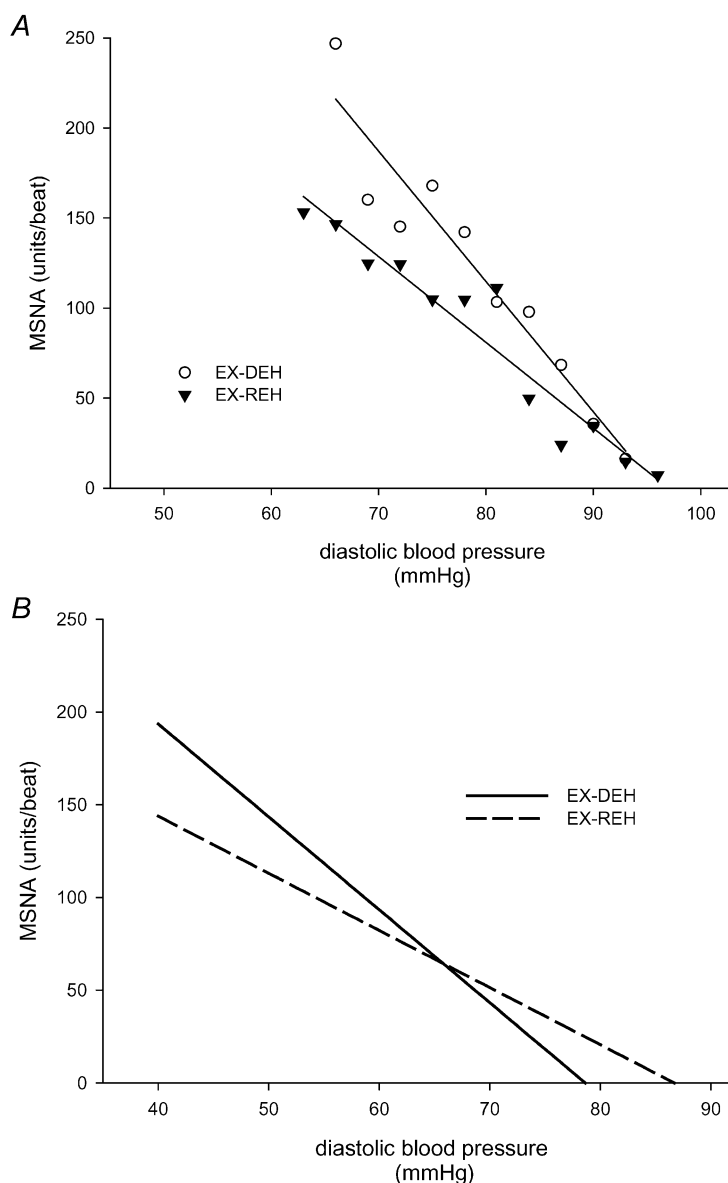
able to minimise that fall in pressure using a dextran infusion. However, the progressive increase in HR during exercise was not different between the control and infusion conditions. As in the present study, the internal body temperature increase was similar between control and volume-infusion conditions, suggesting that body temperature played a role.

We also report here that cardiac baroreflex sensitivity is significantly decreased after exercise dehydration. This finding is strikingly consistent among subjects. We had hypothesised that baroreflex sensitivity would increase in the exercise-dehydrated condition; this would have suggested that the baroreflex acts to offset the orthostatic intolerance seen with dehydration. Although we did not assess orthostatic tolerance directly, our finding that baroreflex sensitivity decreased suggests that the baroreflex contributed to, rather than offset, the orthostatic intolerance seen in this condition.

A decrease in cardiac baroreflex sensitivity is not what would be expected based on previously observed influences of CVP on arterial baroreflex responses. Changes in CVP have been shown to modify the arterial baroreflex control of HR (Pawelczyk & Raven, 1989; Shi *et al.* 1993; Crandall *et al.* 1994). For example, a decrease in CVP had the effect of augmenting cardiac baroreflex sensitivity (Pawelczyk & Raven, 1989; Crandall *et al.* 1994). In the present study, CVP was decreased by the exercise-induced dehydration such that if anything, the foregoing CVP effect would tend to reduce the difference in baroreflex sensitivity between the euhydrated and dehydrated states. Moreover, restoration of volume (and increasing CVP) with saline infusion in the present study did not immediately restore cardiac baroreflex sensitivity post-exercise. Our data therefore suggest that the combination of exercise and dehydration have influences on the arterial baroreflex control of HR that are distinct from those observed previously with lower body negative/positive pressure and prolonged bed rest.

The use of sequential administration of nitroprusside and phenylephrine elicits responses that involve both carotid and aortic baroreceptor populations; thus we cannot conclude from the present data whether one or the other set of baroreceptors were primarily responsible for the changes observed. Furthermore, although we were able to assess a robust linear gain in all cases, in most subjects we were not able to assess the entire sigmoid range of the baroreflex response. Therefore, we cannot draw conclusions relative to the influence of exercise-induced dehydration on the operating range of the response.

In contrast to the cardiac baroreflex, the control of MSNA in the present study followed a pattern that was consistent with the idea that changes in CVP have reciprocal effects on arterial baroreflex responsiveness (Pawelczyk & Raven,

1989; Shi *et al.* 1993). That is, the volume infusion carried out in protocol 1 decreased resting MSNA and tended to decrease the sensitivity of the arterial baroreflex control of MSNA. Our data are also consistent with those of Kimmerly & Shoemaker (2002), who concluded that hypovolaemia (achieved by administration of a diuretic) was associated with higher levels of MSNA for any given decrease in CVP during low-level lower body negative pressure.

In this context, we note that a limitation of the present study was that we were not able to directly compare MSNA before and after exercise/dehydration. Due to technical limitations associated with repeated microneurographic recordings, we chose to limit our recording to the post-exercise conditions. Thus we designed our study to examine the influence of volume on MSNA in the post-exercise dehydrated state. We also note that the values we observed for sensitivity of baroreflex control of MSNA are in the range of those reported previously in similar studies (Halliwill *et al.* 1996*a*; Halliwill & Minson, 2002).

A second potential limitation of the present study relates to CVP and plasma volume changes. The intravenous hydration performed in protocol 1 caused increases in CVP and plasma volume above baseline values. This is probably due to the fact that the volume we reinfused was based on total fluid loss, not on intravascular fluid loss, but the saline was infused into the intravascular space. Although the post-infusion values went above baseline, they did allow us to assess the affect of volume infusion in the dehydrated state on the cardiovascular variables of interest in this study.

Our present data are consistent with the previous work of Halliwill *et al.* (1996*a*,*b*) in that in protocol 1, the operating point for blood pressure regulation was reset to lower levels after exercise. In terms of baroreflex control, however, we noted a decrease in cardiac baroreflex sensitivity after exercise-induced dehydration in protocol 1, whereas with exercise alone, Halliwill *et al.* (1996*a*,*b*) did not note a decrease in cardiac baroreflex sensitivity. Furthermore, Thompson *et al.* (1990) reported that neither hypovolaemia via diuretic nor hypervolaemia via isotonic saline ingestion (in the absence of exercise) altered carotid-cardiac baroreflex control as measured using the neck-cuff method. Thus it appears that exercise in the absence of dehydration, and dehydration in the absence of exercise or other stimuli (e.g. bed rest) may not alter the sensitivity of baroreflex control of HR. Taken together with the present data, we conclude that the decrease in cardiac baroreflex sensitivity seen in the present study was due to a combination of exercise and dehydration. This idea is supported by the fact that the decreases in baroreflex sensitivity were minimised in protocol 2 when saline was given during exercise. Although the overall trend in baroreflex sensitivity was similar to that observed in protocol 1, the changes were more variable and even absent in some subjects in protocol 2.

A potential explanation for these observations is an effect of plasma osmolality on cardiac baroreflex sensitivity. In the present study, plasma osmolality was increased in the EX-DEH and EX-REH trials of protocol 1, suggesting that it has a mechanistic role in the decreased baroreflex sensitivity seen in those trials. Furthermore, osmolality showed a trend ($P = 0.06$) towards an increase in the EX-HY1 and EX-HY2 trials of protocol 2, suggesting that it played a role in the more variable trend towards the decreased baroreflex sensitivity seen after exercise in protocol 2. This idea is consistent with recent data from Bealer (2003), who reported that hyperosmolality decreased baroreflex sensitivity in conscious rats via a central angiotensin-II-dependent mechanism. This possibility that increased plasma osmolality decreases cardiac baroreflex sensitivity requires further testing in humans.

Our finding that saline infusion minimised the hypotension seen after exercise may have important implications for patients with chronic orthostatic intolerance who have a limited ability to exercise due to symptoms during or after the exercise bout. Indeed, fluid ingestion has been shown to improve orthostatic tolerance both in healthy subjects and in patients with chronic orthostatic intolerance (Schroeder *et al.* 2002; Shannon *et al.* 2002). The present findings of altered control of blood pressure and HR post-exercise with changes in hydration status suggest that maintaining hydration is a strategy by which these patients can minimise post-exercise symptom development.

In conclusion, we report that mild exercise-induced dehydration causes an increase in resting HR and a decrease in the sensitivity of baroreflex control of HR. We propose that a non-baroreflex mechanism may have contributed to the increase in resting HR, as this HR was not responsive to increases in volume and pressure caused by volume infusion. These represent potential mechanisms by which exercise in combination with dehydration causes post-exercise orthostatic intolerance and tachycardia in healthy individuals.

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